Measurement of Some Biochemical Parameters Related to Infection with *Plasmodium falciparum* Malaria.

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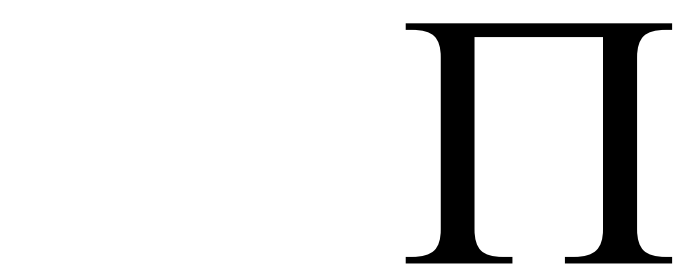

قال تعالى :

((إن الله لا يستحي أن يضرب مثلاً ما بعوضةً فما فوقها فأما الذين آمنوا فيعلمون أنه الحق من ربهم وأما اللذين كفروا فيقولون ماذا أراد الله بهذا مثلاً يضل به كثيراً ويهدي به كثيراً وما يضل به إلا الفاسقين))

سورة البقرة (26).

Dedecated

To the soul of my sister's husband

And to my family

Who were always there by my side

And did every thing for me.

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This work is dedicated to my parents.

ABSTRACT

This work was carried out in the Department of Biochemistry, Faculty of Veterinary medicine, University of Khartoum. Samples were collected from El Duiem province.

Forty seven individuals were selected for this study; all were a adults in El Diuem province. Their age ranged between 20-40 years old. Thirty six of them are malarial patients (19 are males and 17 are females). The other thirty eight individuals were apparently healthy and used as control groups as (18 males and 20 females).The blood samples were taken from the cephalic vein for investigation of malaria parasite, plasma proteins and total IgG.

Total IgG was measured using Radioimmunoassay method (RIA). The effect of sex and degree of parasitemia was considered for all parameters studied.

The result obtained revealed that, total protein and albumin were significantly ($p<0.05$) lower in malaria patients compared to healthy individuals whereas total globulin and IgG appeared significantly increased during infection with *P. falciparum* malaria.

The result obtained showed that, the total proteins , total globulins and IgG were significantly ($p< 0.05$) higher in one cross patients compared to the control, and showed significantly ($p<0.05$) lower values in two cross patients compared to the control, whereas the albumin fraction sowed slightly lower values in patients compared to the control.

ملخص الأطروحة

أجريت هذه الدراسة في كلية الطب البيطري – جامعة الخرطوم -قسم الكيمياء الحيوية . لتقييم أثر الملاريا على بعض بروتينات البلازما والقولوبيولين المناعيIgG .

تم اختيار 74 متطوعاً من منطقة الدويم تتراوح أعمارهم مابين 20- 40 سنة. بصورة عشوائية 36 مصاباً بالملاريا (19 ذكور, 17 إناث) و 38 من الأصحاء (18 ذكور , 20 إناث) للمقارنة كمجموعة تحكم .

جمعت عينات الدم من وريد اليد لاجراء الفحص المجهري للملاريا أولاً ثم قياس مستوى البروتين الكلي والزلال والقلوبيولين الكلي والقولوبيولين المناعيIgG .

وقد اظهرت النتائج الآتي:

1. إنخفاض نسبة البروتين والزلال معنوياً عند مرضى الملاريا.
2. إرتفاع تركيز القلوبيولين الكلي والجسم المناعي (IgG.) معنوياً لدى مرضى الملاريا .
3. عدم تأثر البروتين الكلي والزلال بصورة ملحوظة عند زيادة درجة الإصابة.
4. إنخفاض مستوى الزلال معنوياً عند مرضى الملاريا وكانت النتائج أكثر معنوية عند زيادة درجة الاصابة .
5. عدم تأثر البروتين الكلي والقلوبيولين والزلال بالجنس عند الإصابة بالملاريا.
6. ارتفاع مستوى القولوبيولين الكلي لدى المصابين بالملاريا وكانت النسبة أكثر معنوية في الاصابات الخفيفة.
7. ارتفاع نسبة القولوبيولين المناعي (IgG) معنوياً عند الاناث مقارنة بالذكور.

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Introduction

Malaria remains major problems in many parts of the world are affected annually, and about three million, mostly children, die of falciparum malaria each year. In (1880), Charles Louis Larveran discovered and described malaria parasite in human blood. Sir Ronald Ross's discovery in (1897) of pigmented cysts on the stomach wall of an Anopheles mosquito,in Secunderabad India, revealed how the parasite infectes man. Grassi, Bignami, and Bastianellin in Italy described the cycle of human malaria parasites in Anopheles mosquitoes (Simini, 1999).

The most common causing malaria in humans is *Plasmodium falciparum*. Although the majority of cases are relatively mild, the parasite may also cause sever and life threating malaria, involving sever anaemia hypoglycaemia, renal failure, multiple convulsions and coma (Marsh, 1999).

In the Sudan, the endemicity of malaria varies from holoendemic in the South to hypoendemic in the North. In irrigated areas, transmission ranges form seasonal to perennial and the endemicity ranges from mesoendemic to highperendemic. *P. falciparum* is the dominant species of malaria parasite in Sudan, it causes over 90% of malaria infections in Eastern Sudan close to Ethiopian borders; whereas *P. malariae* is reported mainly from Southern Sudan and *P. ovale* is sporadic (El Gadal, 1990).

In view of recent demonstration that malaria is characterized by moderate decrease in total protein of blood serum at the expense of reduce levels of albumin and increased concentration of globulin (Abdelgadir, 2002; Abdalgalil, 2003 and Abushama, 2003).in this assay an attempt is made to investigated total protein, albumin and immunoglobulin IgG

contents of sera from individuals with P. falciparum malaria and to be compared with healthy individuals.

Chapter One

Literature Review

1.1 Classification of Malaria Parasites of Medical Importance according to Levine et al, (1980).

Phylum:Apicomplexa (Levine, 1970)

Class: Sporozoa (Leukart, 1879)

Sub Class: Coccidia (Leukart, 1879)

Order: Eu coccidiida (Basco,1910)

Sub order: Haemosporina (Danilesky, 1885)

Family:Plasmodiidae (Mensil, 1903)

Genus: Plasmodium (celli, 1885)

Wide spread species *Plasmodium falciparum* (Welch, 1879)

Plasmodium vivax (Grassi and Feletti, 1890)

Plasmodium malariae (Laveran, 1881)

Less wide spread species *Plasmodium ovale* (Stephens, 1922)

1.2 Plasmodium falciparum

The name *falciparum* comes from the characteristic sickle shape of the gametocytes of this species .This is the most highly pathogenic of all the plasmodia and hence the name malignant tertian or pernicious malaria

infection. The disease has a high rate of complications and unless treated, is often fatal.

The species is responsible for the largest number of malaria cases world wide (80 percent) and is deeply entrenched in tropical Africa and some parts of Asia. It is limited to the tropical and subtropical regions, because at temperatures below 20°C, its development in the mosquito is greatly retarded.

The sporozoites are sickle shaped. The tissue phase consists of only a single cycle of pre-erythrocytic Schizogony. No hypnozoites occur, the mature liver schizont releases about 30.000 merozoites. They attack both young and mature erythrocytes (Bruce, 1985)

1.3 Transmission and Life Cycle

The main factors which influence the epidemiology of *falciparum* malaria are the intensity of transmission and the immune response of the infected person.

Malaria transmission in an area may be

Stable

Un stable

1.3.1 Stable malaria

In areas where malaria transmission is stable, transmission occurs for at least 6 months in a year and is intense. Children suffer repeated attacks from the age of a few months. Those who don not die, have a substantial immunity by the age of five or six years. When immunity is established, patients may still suffer attacks of malaria but these are comparatively mild

and last for only a few days. Older people are little affected. There is little variation in the incidence of malaria from year to year although there may be marked seasonal fluctuations, particularly in children (Schimdt, 1981).

1.3.2 Un stable malaria

In areas where malaria transmission is un stable; there are marked changes in transmission from one season to another and from one year to the next. The transmission season is short and infection of any one individual is comparatively infrequent so that immunity is unable to reach a high level. When an out break of malaria occurs, usually following explosive breeding of mosquitoes, it does so in the form of an epidemic with people of all ages being susceptible and after severely at risk. . *P. falciparum* is transmitted by many species of female Anopheline mosquitoes. It can also be transmitted by transfusion of infected donor blood or by injection through the use of needles and syringes contaminated with infected blood. Very occasionally congenital transmission occurs, usually when a mother is non-immune (Schimdt, 1981).

1.4 Life cycle of P. falciparum

The *plasmodium* life cycle is complicated, comprising several development stages in both the vertebrate host and in the initiated by inoculation of sporozoites from the salivary glands of an infected Anopheles mosquito. Within minutes the sporozoites invade hepatacytes in the liver and multiply for a week or two when thousands of merozoites are released from the ruptured cells, ready to invade erythrocytes and initiate the erythrocytic life cycle. After invasion of erythrocytes the parasite undergoes mitotic

division and matures into a schizont containing 32 merozoites. The erythrocytes then repture and release the merozoites into the blood stream, causing clinical symptoms such as fever and anaemia. The merozoites quickly reinvade new erythrocytes and the cycle is negated. Some of the parasite will occasionally develop into female or male gametocytes, the stage infective to the mosquitoes. In the mosquito midget the gametocytes will take place. The resulting zygotes will form OOcysts and differentiate into sporozoites that finally migrate to the salivary glands of the mosquito.

Infection is mostly characterized by a non-specific febrile illness. Some degree of anaemia is also developed in children. In cerebral malaria, when the parasites invade the brain erythrocytes, a variety of neurological manifestations include impairment of consciousness, seizures, cranial nerve neuropathics develop after the infection (Newton and Warrell, 1998).

1.5 Malaria in Sudan

Malaria is one of the leading causes of illness and death in the tropical and subtropical region of the world it is the most prevalent of all infections disease (WHO, 1997). The endemicity of the disease in Sudan range from holoendemic in the south to hypoendemic in the North. This follows, more or less the natural geographical zones, as well as the natural transmission in the amount of the rain fall in the country, which is zero in the far north to over 800 mm in the South. Generally malaria transmission season in the South of the country occurs during and after the rainy season (Abdel Rahim, 1998).

All four plasmodium species have been reported in the country but the predominant species is *P. falciparum* which accounts for about 90% of the reported malaria cases (Omer, 1978; Taha and Broadhead, 1986). Followed

by *P. vivax* and *P. malariae*. Previous unpublished reports, recorded *P. ovale* in rare cases in Sennar and Western Sudan. Recently *P. vivax* and *P. ovale* have been reported in Khartoum (El Sayed, 1998).

1.6 Socioeconomic and behavioral factors

Poor people are at increased risk both of becoming infected with malaria and of becoming infected more frequently. Moreover, poor socio-economic status and socio cultural factors together with human behavior such as the type of housing, sleeping habits, poor knowledge about the disease and treatment. Seeking behavior play an important role in maintaining high degrees of malaria transmission (Sharma *et. al*, 2001).

In Sudan, malaria accounts for 25.7 % of the hospital admissions and 15.9 % of the total deaths (FMOH, 2001). Displaced admissions in Southern Sudan suffer from a high incidence and prevalence of malaria. Moreover, population movement simultaneously with the spread of *P. falciparum* resistant strains had further aggravated the problem (Guthmann, *et. al*, .1996).

1.7. The Blood

Blood performs many functions in the body. The main function of the circulation blood is to carry oxygen and nutrients to the tissues and to remove carbon dioxide and waste products from the tissues. In addition, blood transports other substances (e.g. hormones) from their sites of production to their sites of action and white blood cells and platelets to where they are needed. Blood also aids in the distribution of water, solutes, and heat and thus contributes to homeostasis, the maintenance of a constant internal body, environment. Blood consist of red blood cells, white blood cells, and platelets suspended in a complex solution (plasma) of gases, salts, proteins, carbohydrates and lipids. The circulating blood volume, accounts for about 7% of body weight. Approximately 55% of the blood is plasma, the protein contain is 7g /dl (Robert, 1999).

1.7.1 Plasma Proteins

Proteins perform a number of essential functions in all forms of life. The name in fact, is derived from the Greek protos, meaning “first” or “primary” proteins serve a structural role within the cell (cytoskeletion) and within the connective tissue and skeleton of the whole organism. Proteins, also function, interalias, as catalysts. About 19% of the most abundant protein in humans. Proteins are polymers of α – amino acids. There are 20 amino acids that are genetically encoded and that serve as precursors for protein bio synthesis on ribosomes. Some of these amino acid residues are modified or dervatited after biosynthesis (Post translational modification). (Murry , 2000).

1.7.2 Albumin

Albumin is present in highest concentration in the serum. It is synthesized in the liver. Albumin has two well known functions. One is the contribution albumin makes to colloid osmotic pressure of the intra vascular fluid; due to it is pressure, which maintains the appropriate fluid in the tissues. The other prime function is its ability to bind various substances in the blood. For example albumin binds bilirubin, salicylic acid, fatty acids, calcium, magnesium ions, cortisol, and some drugs.

Decrease concentration of serum albumin may be caused by the following

1 An inadequate source of amino acids, which is seen in malnutrition and muscle wasting diseases.

2 Liver disease resulting in the inability of hepatocytes to synthesize albumin. The increase in globulins that occurs in early cirrhosis, however, will balance the loss in albumin to give a total protein

concentration within acceptable limits.

3 Gastrointestinal loss as interstitial fluid leaks out in inflammation and disease of the intestinal mucosa.

4 Loss in the urine in renal disease. Albumin is normally excreted in very small amounts. This excretion is increased when the glomerulus no longer functions to restrict the passage of proteins from the blood. (Campbell, 1999).

1.7.3 Total globulins

Globulins are protein molecules that are insoluble in plain water but soluble in salt water. The serum globulins are a heterogeneous , complex mixure of protein molecules that are frequently designated as ά β or γ-globulins.Total globulins account for about 50 percent of total protein of normal serum. The predominant globulin in most animals, such as man, monkey, and rabbit, has a molecular weight between 150000 and 160000 Da, but other globulins with molecular weights of 200000, 400000 and 900000 and also found in grater or less concentration. Normal globulins assets in the maintenance of blood and tissue osmotic relations and take part in cell nutrition (Mohan, 1998).

Abdalgalil, (2003) reported that the values of total globulins showed significantly ($p<0.05$) higher levels in patient compared to the normal individuals, while the albumin was of similar levels in one cross patients and controls.

1.7.4 Effect of malaria on total Protein and Albumin

In a study camed by Alumanah (2000), serum protein levels were determined in 158 malarial patients infected with Plasmodium falciparum. In

contrast to an earlier reported protein deficiency during malarial infection, the results obtained from this study showed no significant change. The significance ($P< 0.05$) of the results only related to excessive protein catabolism in fever.

In patients with uncomplicated plasmodium falciparum infection cytokine-mediated serum protein levels as c- reactive protein (CRP). Cocruloplasmin (COE), beta 2- microglobulin (B2M), alpha 1- acid glycoprotein (AAG), alpha 1-antitrypsin (AAT), haptoglobin (HPT), pre albumin (PRE), retinol binding protein (RBP), albumin (ALB) and transferrin (TRF) were measured in an endemic area of the Amazonian rain forest. Semi-immune (SI) and nonimmune (NI) patients were investigated. In both patient groups the serum concentrations of CRP, COE and B2M were elevated on admission. In addition AAG and AAT concentrations were increased in NI patients compared to control subjects. Significantly lower serum concentrations of HPT, PRE, RBP, ALB and TRF were seen in both patient groups during the acute phase of the disease, and were more pronounced in NI patients. After a 28 day follow-up. AAT and HPT, AAT and B2M were still significantly altered in NI patients (Graninger, 1992).

Mishra, (1992) found that serum total protein and albumin were significantly decreased but those were considered more as indicator of acute phase response. Liver cell necrosis was observed in one patient, and oedema and mononuclear cell infiltration in two patients. Though hepatomegaly and mild elevation of enzymes can be observed in a significant proportion of patients, involvement of liver leading to acute hepatitis or liver cell necrosis is a relatively uncommon complication in *P. falciparum* malaria.

1.8. Immunity against Malaria

Immunity against malaria is complex partially due to parasite antigenic variation expressed at stages of the parasite life cycle. However, it became clear that both specific and non specific mechanisms are involved. A considerable part of the non-antigen specific immune response contributes to the natural defense against malaria (Druilhe and Pergnon, 1994).

In areas with stable endemic *P.falciparum* malaria, parasitaemia is most common in young children, and the incidence of parastitemia declines steadily with age. This has long been interpreted as evidence of gradual acquisition of specific immunity to malaria .The acquisition of such immunity is slow, develops gradually after many years of exposure. The precise timing of events depends on local patterns of malaria transmission and levels of endemicity .Therefore, in the highly endemic malaria areas where the adult population posses acquired immunity, children present the major susceptible population and source of malaria, since they don't have acquired immunity, and their parasitaemia is usually heavy (Marsh,1996) .

1.8.2 Humoral Immunity

In residents of endemic areas, malaria infection induces strong humoral immune responses, involving production of predominantly IgM and IgG also of other immunoglobulin 1so types. While a large proportion of this immune globulin is non-malaria specific, reflecting polyclonal B-cell activation, up to 5% or more represent species – as well as stage-specific antibodies reacting with a wide variety of parasite antigens. Passive transfer of IgG from immune donors already suggested long ago that antibodies may be protective (Orago, et .al., 1991 and Mc Gregor, 1963) by reducing parasitienia and clinical disease.

1.8.3 Antibodies

Malaria infection induces polyclonal and specific immunoglobulin production. Al though antibodies of different isotypes may have protective functions, IgG is most important in this respect. In protected individuals, cytophilic antibodies of IgG1 and IgG3 isotypes have frequently been found to prevail (Bouharoun_Tayoun , 1992 and Sarthou, 1997).

The ratio of IgG1 to IgG3 antibodies appears to be highest in subjects, whose antibodies are also most efficient in parasite neutralization invetro, supporting the functional relevance of these findings (Shi, 1999). Significant elevations of IgG3 antibodies in certain populations and associated with disease episodes have been reported (Aribot, 1996 and Rzepezyk, 1997). However elevated concentrations by IgG2 antibodies may also be associated with deceased risk of *P. falciparum* infection. This has been seen in certain individuals whose monocytes carry especial allelic variant of a Fcγ receptor (RIIA) having the capacity to bind this normally not cytophilic immunoglobulin subclass (Aucan, 2000).

1.8.3 Antibodies Structure and Function

Antibodies constitute a group of globular serum proteins called immunoglobulin (Igs). A typically antibody molecule has two identical antigen- binding sites specific for eptope that provoked its production. Each molecule consists of four polypeptide chains, two identical heavy chain and two identical light chains, joined by disulfide bridges to form a Y shaped molecule. At the two tips of the Y-shaped molecule are the variable regions (V) of the heavy and light chains, so named because the amino acid sequences in these regions vary extensively from antibody to antibody, a heavy –chain V region and a light-chain V region together form the unique contours of an antibody's antigen – binding site. The interaction between an

antigen-binding site and its epitope resembles an enzyme-substrate interaction.Thus is by multiple noncovalent bonds formed between chemical groups on the respective molecules. The power of antibody specificity and of antigen –antibody interaction has been known by the use of antibodies in laboratory research clinical diagnosis, and the treatment of diseases. In particular, the technology for the production of monoclonal antibodies is an extraordinary contribution to biomedical science. While the antigen-binding sites responsible for an antibody's ability to identify a specific epitope as an antigen, the tail of the Y shaped antibody, formed by the constant regions (c) of the heavy chains, is responsible for its distribution in the body and for the mechanisms by which it mediates antigen disposal.There are five major types of heavy –chain constant regions, and these determine the five major classes of antibodies: IgM, IgG, IgA, IgD and IgE. IgMs are the first circulating antibodies to appear in response to an initial exposure to an antigen. Their concentration in the blood declines rapidly. This is diagnostically useful because the presence of IgM usually indicates a current infection by the pathogen causing its formation. IgM consists of five Y shaped monomers arranged in pentamer structure. The numbers of antigen-binding sites make it very effective in agglutinating antigens and in reactions involving complement. IgM is too large to cross the placenta and does not confer maternal immunity. IgG is the most abundant of the circulating antibodies. It readily crosses the walls of blood vessels and enters tissue fluids. IgG also crosses the placenta and confers passive immunity from the mother to the fetus. IgG protects against bacteria, viruses and toxins circulating in the blood and lymph, and triggers action of the complement system. IgA is produced primarily in the form of two Y-shaped monomers (a dimmer) by cells abundant in mucous membranes. The main function of IgA

is to prevent the attachment of viruses and bacteria to epithelial surface. IgA is also found in many body secretions, such as saliva, perspiration and tears. Its presence in colostrums (the first milk of a nursing mammal) helps protect the infant from gastrointestinal infections. IgD antibodies do not activate the complement system and can not cross the placenta. They are mostly found on the surface of B cells, probably functioning as an antigen receptor required for in tiating the differentiation of B cells into plasma cells and memory B cells. IgE antibodies are slightly larger than IgG molecular and represent only a very small fraction of the total antibodies in the blood. The tail regions attach to receptors on most cells and basophiles and, when triggered by an antigen, cause the cells to release histamine and other chemicals that cause an allergic reaction (Roitt, *et. al*., 1993).

1.8.4 Effect of antibody on malarial parasites

Antibody is the most important part of the immune response against those parasites that live in the blood stream, such as African trypanosomes and malarial parasites, where as cell-mediated immunity is activate against those like leishmania that live in the tissues. Antibody can damage parasite by helping in phagocytosis, activate complement or block their entry into their host cell and so limit the spread of infection. Malaria parasites within the red cell may be destroyed by some recreated products of activated macrophages, including hydrogen peroxide and other cytotoxic factors (Roitt *et. al*, 1993). There is no clear pattern of association between IgG subclass and protection against malaria in different endemic areas. The IgG protection is not yet conclusive and this might reflect the varied roles of total anti – *P. falciporum* IgG response during the course of infection. However, the most consistent finding in malaria studies is the blocking role of IgG4

against other isotypes which indeed represent the lowest percent of IgG response. As such, preliminary investigation of the role of total IgG and IgG4 in a certain community may give an idea of the role of other isotypes (Abushama, 2003).

Luty *et al*, (2000) measured sporozoite and total parasite antigen-specific IgG and IgM antibodies before and after treatment in matched groups of Gabanese children who presented with either mild or sever *P. falciporum* malaria. They investigated the influence of various parameters on these antibody responses, including clinical presentation, age, and post-treatment reinvention profiles. IgG but not IgM responses were strongly influenced by both clinical and parasitological status. IgG responses to the repeat region of the circumsporozoite protein, which were low at admission particularly so in those with sever anemia, increased after treatment but showed no association with either age or reinvention profiles. Total parasite antigen – specific IgG responses were strongly influenced by parasitological status, and also differed significantly when segregated according to clinical status at admission, age, and reinvention histories. Most notably, anti-parasite IgG responses measured when children were parasite- free where higher and a good indicator of recent reinventions in those who presented with mild rather than with sever malaria. The profile of responses in the latter group suggests some immune system dysfunction, which may reflect the induction of tolerance to parasite antigens.

The kinetics of indicators of lymphocyte activation was determined in non- and semi immune patients with uncomplicated *P. falciparum* infection and in control subjects in Acre, Brazil. Delayed type hyper sensitivity (DTH) to seven recall antigens was weakest in non immune patients; both patient

groups differed significantly from controls on admission ($P < 0.001$ for both) and improved considerably after clindarycin therapy (Kremsner et, al., 1990).

Total serum IgG and IgM, but not anti malarial antibodies, were highest in non immune patients compared with semi immune patients and controls during acute malaria. Immunoglobulin levels normalized after chemotherapy. A striking decrease of $CD4^+$ peripheral blood lymphocytes, normalizing after chemotherapy, was more pronounced in non immune patients. A slight increase in interleukin -2 receptor (IL- 2R). Soluble plasma IL- 2R was significantly elevated in them ($P < 0.001$) and to a lesser extent in semi immune patients. These concentrations in plasma ($P < 001$) during the acute phase of malaria, suggesting pronounced general immuno suppression in non-immune malaria patients (Kremsner *et, al*., 1990).

Transfer gamma globulin from immune adult to a child infected with plasmodium falciparum caused a sharp drop in parasitaemaea. Specific antibody acts at the merozoite stage in the life of the parasite and prevents the initiation of further cycles of multiplication in the blood. The development of agametocytes from existing intracellular forms is unaffected. The presence of immune serum blocks the continued increase in number of *P. knowless* (malarial parasite of monkeys), as measured by incorporation of ^{3}H-leucine it stops multiplication at the stage after schizont rapture by preventing the releases merozates from invading fresh red blood cells. The inhibitory activity of the immune serum can be reduced by prior absorption of the specific antibody with the Schizonts.

Chapter Two

Materials and Methods

2.1 The Study Area

The area of the study was El-Dueim,, is located on the West Bank of the white Nile about 150 kilometers south of Khartoum and it is linked to the national capital by a paved high way .It extends for 5 kilometers from south to north .According to the 1993 census the total population of EL-Dueim was 76.336 . The main tribes of the area include the Gaafra, Hassaniya, Shaiqiya and Kurtan. The main occupation for the majority of the population in the study area is agriculture, although there are also merchants, many governmental employees (most of who live in a separated neighbour hood) and labourers. The majority of the houses are built with mud, the rest with brick and with straw and mud. Wall material appears to diffrentiate between types of resedence .Houses in the town centre and government houses, especially for high rank employees are used to be built by brick. The main of the houses in third and fourth class are built by mud. Houses built by straw and straw and mud are found only in fourth class residence (Sharief, 1990).

2.2 Subjects

Forty seven individuals were selected for this study; all were a dults in El Diuem province. Their age ranged between 20-40 years old. Thirty six of them are patients, infected with *Plasmodium falciparum* malaria, (19 are males and 17 are females). The other thirty eight individuals were apparently healthy and used as control grouped as (18 males and 20 females).

2.3 Preparation of sample

Blood sample were collected from controls and patients using sterile syringes and then allowed to clot at room temperature, serum then separated in cuvate containers and kept at – 20°C until analyzed for total protein, albumin and immunoglobulin IgG concentration, using chemical and immunological methods.

2.4 Preparation of Giemsa's Stain

One liter of the stock solution of Giemsa stain was prepared from 7.6 Giemsa powder, 500 ml from each of pure glycerol and methyl alcohol. The stain was prepared mixing alcohol glycerol and those small quantities of Giemsa powder were added gradually until most of the powder dissolved and the solution was stored in brown bottles in a dark place. The staining solution was prepared freshly by mixing 10 ml of Giemsa stock solution with 90 ml buffered water providing 10% conciliation technique for staining blood smears. The thick and thin blood films were taken on the same slides and the thin film was fixed with methyl alcohol for 30 seconds.The slides were stained with the diluted 10% Giemsa stain for 10 minutes and then the slides were washed with tap water and dried for examination.

2.5 Examination of blood films

Thick and thin blood smears were examined, 100 microscopic fields under an oil immersion lens. The thick films were checked to detect parasites and quantify the parasitaemia while the thin films to distinguish infecting species.

2.6 Determination of the parasite density

It is based on the number of parasites per µL of the blood in a thick film counted in relation to a predetermined number of 8000 leukocytes per µL simple mathematical formula was used

Parasites per µL= No. of parasites x 8000/ No. of leucocytes.

The number of the parasites was counted against leucocytes and after 200 leucocytes, if 9 or less parasites were detected; the count was continued until 500 leucocytes and recorded as parasites per 500 leucocytes.

To determine the result of parasite density by crosses, we can use the formula:

+: one cross : (1-10 asexual form of parasites per 100 fields).

++: two cross: (1-99 asexual form of parasites per 100 fields).

2.7 Biochemical investigation

Kits used in biochemical measures of total protein and Albumin were obtained from linear chemicals Laboratory in Spain.

2.7.1 Serum Total Protein

Serum total protein was measured using the kits (Linear chemicals laboratory in Spain). The method used was colorimetric determination based on the principle of Biuret reagent (cupric ions in alkaline solution). The intensity of the blue color was proportional to the protein concentration. The absorbances were measured at wave length 546 nm.

Total protein was calculated according to the following equation Protein in g / 100 ml = Absorbance of the sample / Absorbance of the standard*standard concentration.

2.7.2 Serum Albumin

Serum albumin was measured using the kits (Linear chemicals laboratory in Spain). The method used was colorimetric determination based on the principle of Bromo cresol binds quantitatively to the indicator BCG forming a green colored complex.

2.7.3 Serum Globulins

Serum globulins were found by subtracting total albumin from total serum protein.

2.8 Measurement of immunoglobulin IgG By the radio immunoassay (RIA)

The islets test RIA kit (Beijing Atom High-tech co., LTD .China) was used for the invetro detection of circulating human IgG Auto antibodies specific against *P. falciparum* malaria.

2.8.1 Principle of the method

The radioimmunoassay method depends on the competition between iodine -125 labeled IgG and IgG in standards or in specimens to be assayed, for a fixed and limited number of IgG antibody binding sites, after the incubation, the amount of iodine 125 (I^{125}) labeled IgG bound to the antibody is inversely related to the amount of IgG present in the sample. The separating agent is solid phase second antibody micro particles. Separation of the antibody bound fraction is effected by centrifugation and decanting the supernatant. By measuring the proportion of I^{125} labeled IgG bound in the presence of standards containing various known amounts of IgG, the concentration of IgG in known samples can be interpolated .

2. 8. 2 Reagent

Reagent A (Human IgG standards)

A: 1 , B:2 , C:5 , D:10 , E: 20 , F:50 µg ml

Reagent B Iodine 125.IgG (red)

Reagent C Antibody (blue)

Buffer

2.8.3 Procedure

1. Preparation of samples

Sample and controls were diluted 1: 2601 with buffer prior to assay (20µ l serum + 1ml buffer and then 20 µ l diluted serum + 1 ml buffer).

2. Preparation of working solution

- Reagent A was diluted by adding I.0 ml distilled water before used.
- Reagent B was diluted by adding 11 ml buffer before used.
- Reagent C: 22 ml was taken and shacked thoroughly before used.

3. Analytic procedure

- All tubes were labeled duplicate and arranged in the assay rack.
- 100µl of human IgG standard, buffer and unknown serum samples to prelabelled were pipetied.
- 200µl of anti – IgG antibody were added in all tube except of total count.

- All tubes were mixed well and incubated for 1 hour at 37̊c at which a competition reaction between the lablled and cold antigen took place, RIA reaction.
- 1 ml of wash buffer was added to all tubes except of the total count.
- All tubes were centrifuged at 2500xg for 15 min.
- The supernatant was decanted and the deposit was counted for 60 seconds in Gamma counter.
- The result was analyzed using the WHO immunoassay programs (A5.2) soft ware prepared by Edwards (1988).

2.8.4 Calculation of results

Results were calculated using ling-lag plotting.

1- Express the counts (B) fir each of the standards and unknowns as a percentage of the mean counts of the zero standards (Bo).

$$B/_{Bo} \quad \frac{\text{B of standard or unknown}}{\text{Bo}} \quad \text{X } 100\%$$

2- Plot the percentage values obtained for the IgG concentration on ling-log graph paper and constructs a standard curve.

3- Read the IgG concentration directly from the curve for each of the unknown samples.

2.8.5 Statistical analysis

Data were subjected to statistical analysis using SPSS computer programs (Statistical package for social sciences).

Chapter Three

Results

3.1 The effect of falciparum malaria on some serum proteins.

3.1.1 Plasma proteins and IgG.

The effect of *falciparum* malaria on plasma total proteins, Albumin, total globulins and total IgG, is presented in (Table 1) , (Fig 1-2) .

3.1.2 Total proteins

The level of serum total proteins was lower in patients group but the difference was not significant compared to that of control subjects.

3.1.3 Albumin

The level of serum albumin in falciparum malaria patients was significantly ($p<0.05$) lower than the control group.

3.1.4 Totalglobulins

In all malaria patients studied the mean values of total globulins showed significantly ($p< 0.05$) higher value compared to the healthy individuals used as control.

3.1.5 Total IgG

The level of total IgG was found to be of significantly ($p< 0.05$) higher values in malaria infected patient compared to the control.

Table (1): The effect of infection with *P. falciparum* malaria on serum proteins and IgG.

Select	Total proteins g/dl	Albumin g/dl	Total globulin g/dl	Total IgG µL/ml
Control (n=38)	6.89 ^{a} ± 0.63	4.6 ^{a} ±0.55	2.28 $_{b}$ ±0.81	3.73 $_{b}$ ±0.90
Patients (n=36)	6.64 ^{a} ±0.50	4.08 $_{b}$ ±0.30	2.58 ^{a} ±0.52	4.50 ^{a} ±1.00

Means within the same columns followed by different letters are significantly different at ($p<0.05$).

N: number of replicates.

Fig 1: The effect of infection with *P. falciparum* malaria on serum proteins.

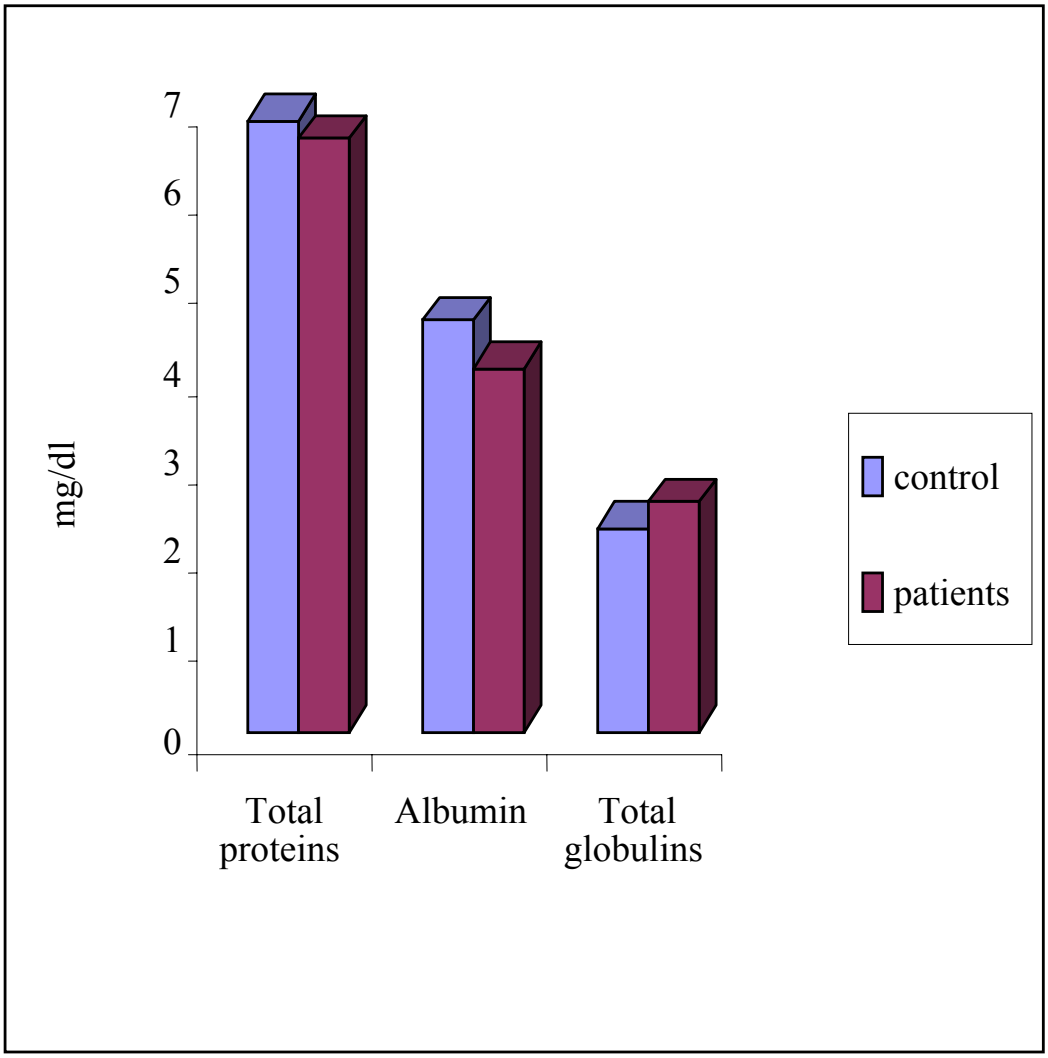
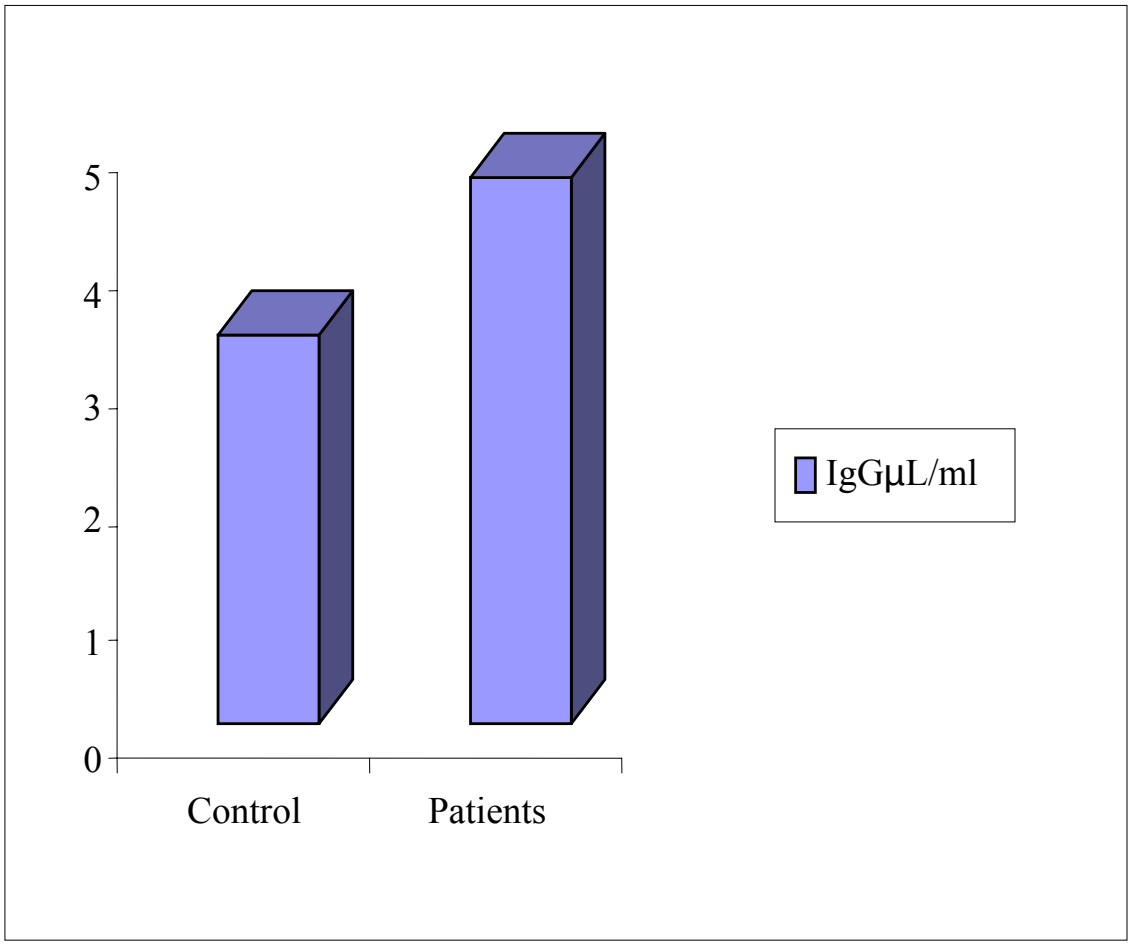


Fig 2: The effect of infection with *P. falciparum* malaria on serum total IgG.



3.2 The effect of sex on serum proteins and total IgG in patient infected with Plasmodium falciparum malaria.

The effect of sex on serum proteins, albumin, total globulins and total IgG in infected patients with *P. falciparum* malaria is presented in (Table2), (Fig 3-10).

3.2.1 Total proteins

The level of serum total proteins is not affected by the sex of the patient, but when females only were compared, the value of total proteins showed significantly ($p<0.05$) higher level in control compared to the infected female. When male and female patients are compared the values of total protein showed more or less the same levels in both sex.

3.2.2 Albumin

Albumin fraction showed significantly ($p< 0.05$) higher level in male control compared to male patients but the difference was not significant for the females. When males and females patients are compared the values of albumin showed significantly ($p< 0.05$) increased levels in males control compared to females.

3.2.3 Total globulins

In male malaria patients studied the mean values of total globulins showed significantly ($p< 0.05$) higher value compared to the healthy individuals. Whereas female patients recorded significantly ($p< 0.05$) lower values compared to normal females. The values of total globulins showed significantly ($p< 0.05$) higher levels in normal females compared to males but the difference was not significant in male and female patients.

3.2.4 Total IgG

The level of total IgG was found to be significantly ($p< 0.05$) higher values in malaria infected female patient compared to female control. This result was also seen when comparing total IgG of male patients and normal males. But when male and female are compared the values of total IgG showed significantly ($p< 0.05$) higher level in female patients compared to the males.

Table (2): The effect of sex on serum proteins and total IgG in patient and controls.

Select	Total proteins g/dl		Albumin g/dl		Total globulin g/dl		Total IgG µL/ml	
Control	male	female	male	female	male	female	male	female
Male (n=18) Female(n=20)	6.76A ^{a} ± 0.74	7.02A ^{a} ± 0.50	5.03 A^{a} ± 0.23	4.23B ^{a} ± 0.48	1.72B $_{b}$ ± 0.69	2.78A ^{a} ± 0.54	4.18A$_{b}$ ± 0.79	3.33B$_{b}$ ± 0.80
Patients								
Male (n=19) Female(n=17)	6.60A ^{a} ± 0.49	6.70A$_{b}$ ± 0.54	4.05A$_{b}$ ± 0.31	4.12A ^{a} ± 0.30	2.60A ^{a} ± 0.58	2.55A$_{b}$ ± 0.44	4.39B ^{a} ± 0.91	4.66A ^{a} ± 1.15

Means within the same columns followed by different small letters are significantly different at ($p< 0.05$).

Means within the same columns followed by different capital letters are significantly different at ($p< 0.05$).

Fig 3: The effect of sex on serum protein in control.

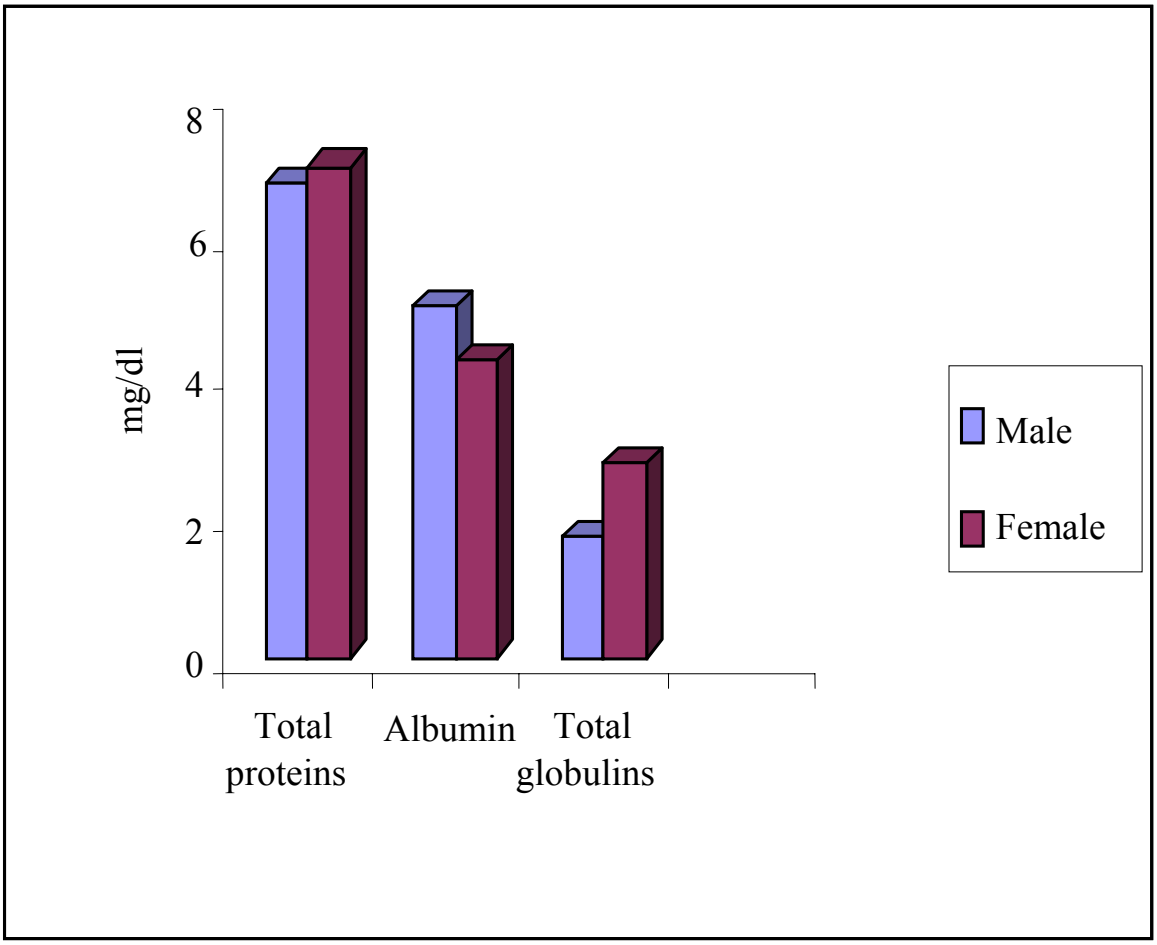


Fig 4: The effect of sex on serum protein in patient with *P. falciparum* malaria.

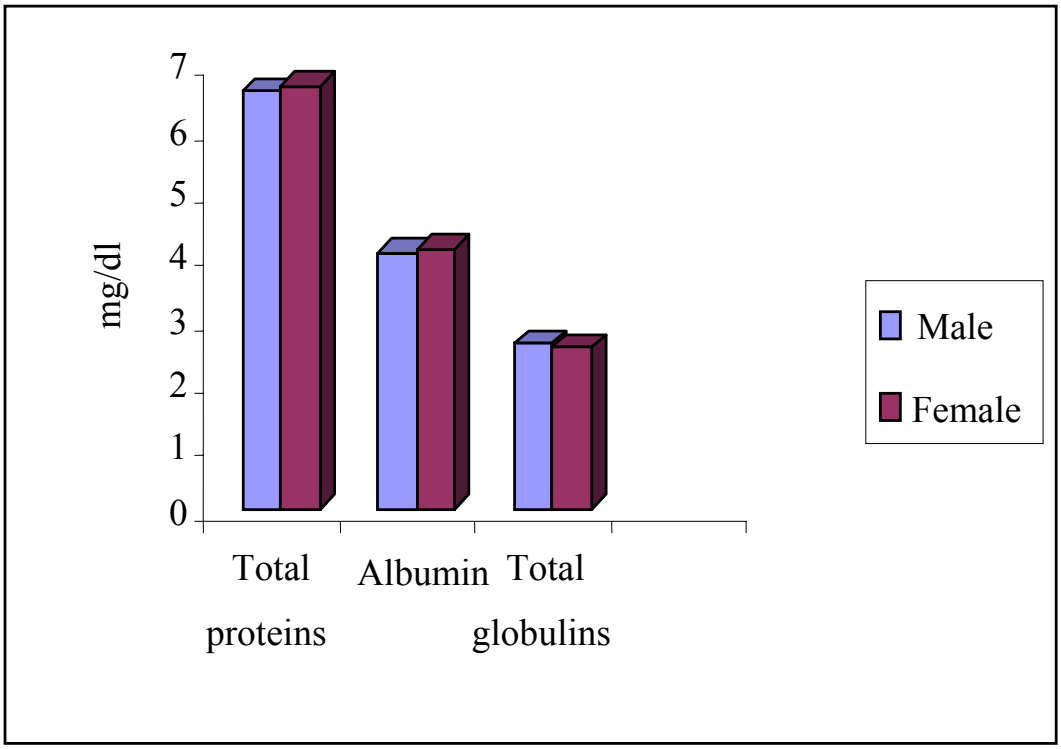


Fig 5: Mean of total protein, albumin and total globulins in male patients.

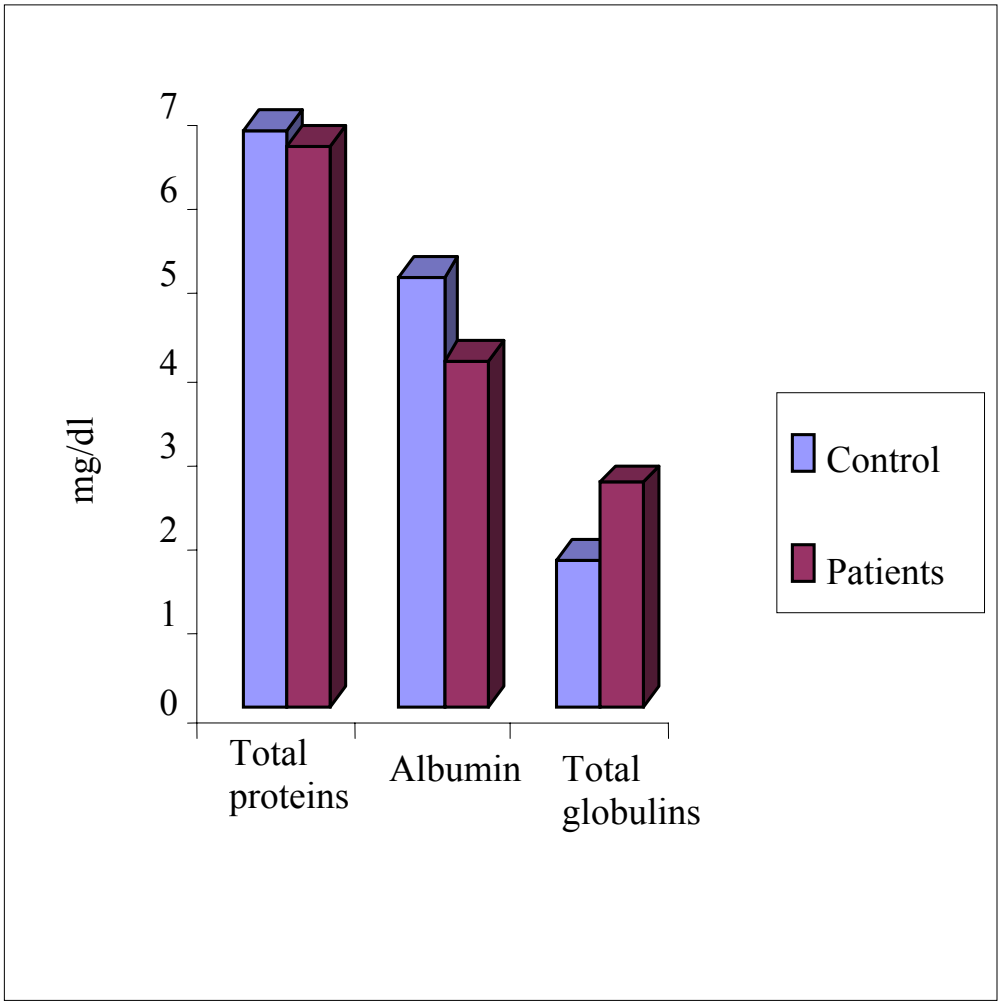


Fig 6: Mean of total protein, albumin and total globulins in female patients.

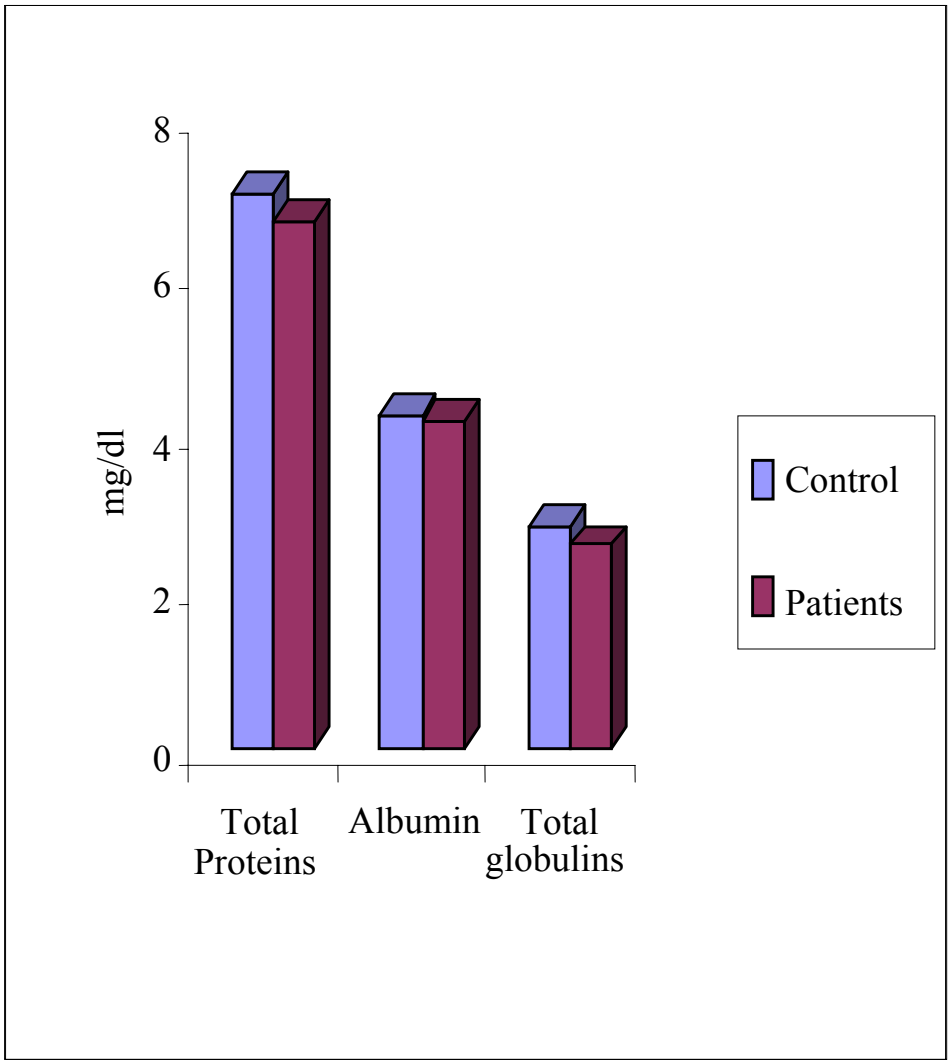


Fig 7: The effect of sex on serum total IgG in controls.

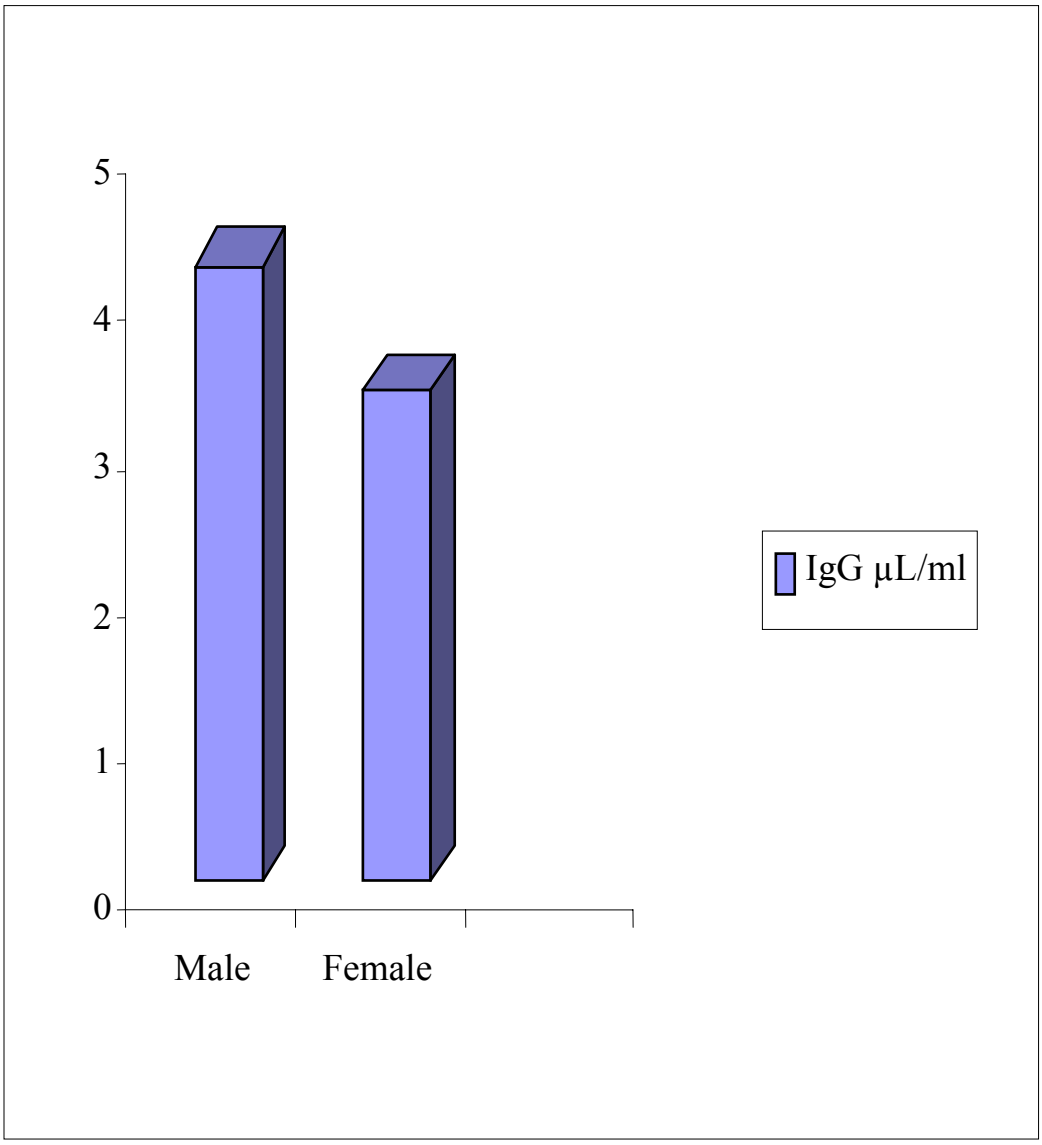


Fig 8: The effect of sex on serum total IgG in patient with p.falciparum malaria

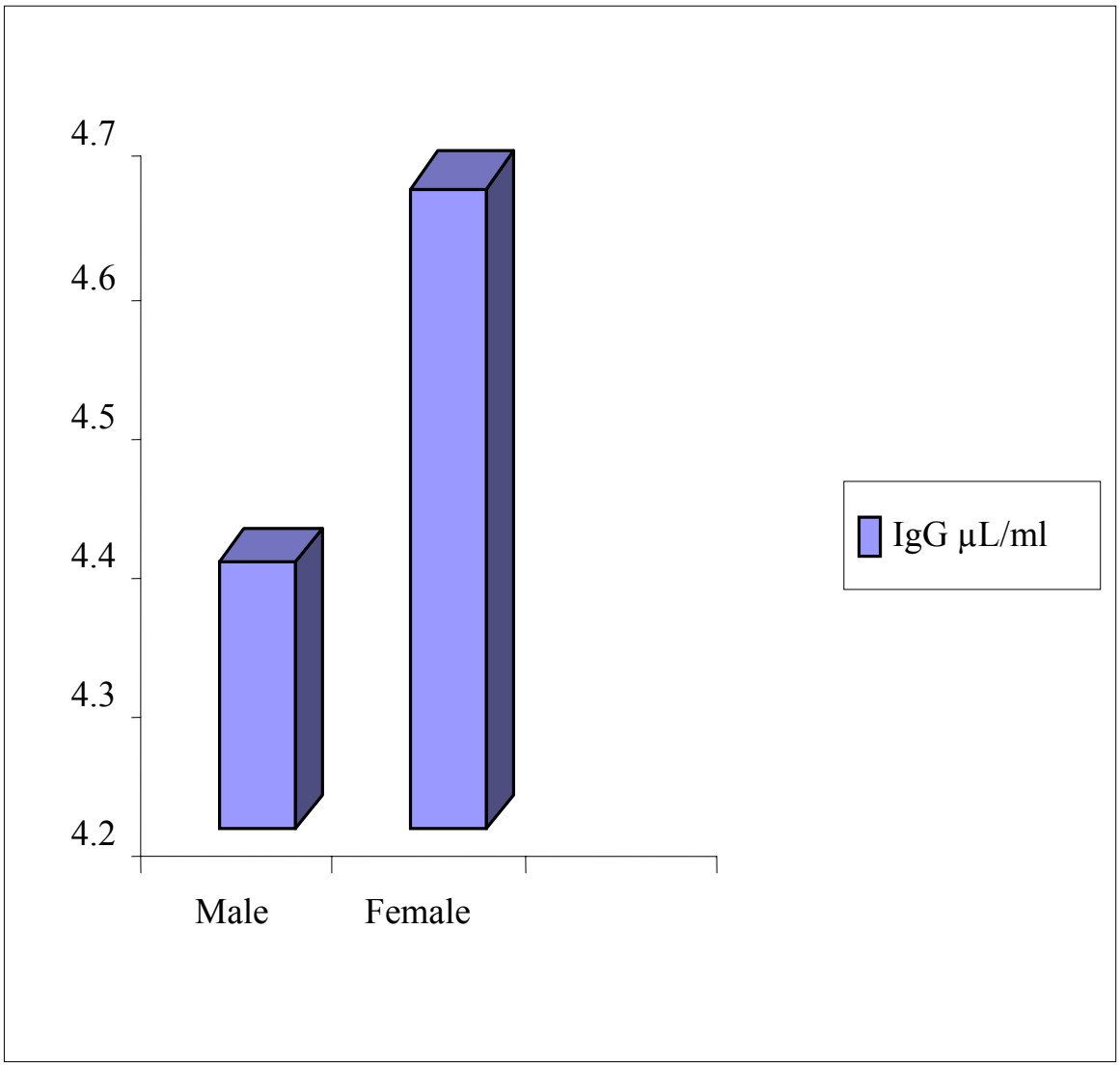


Fig 9: Mean of total IgG in male patients.

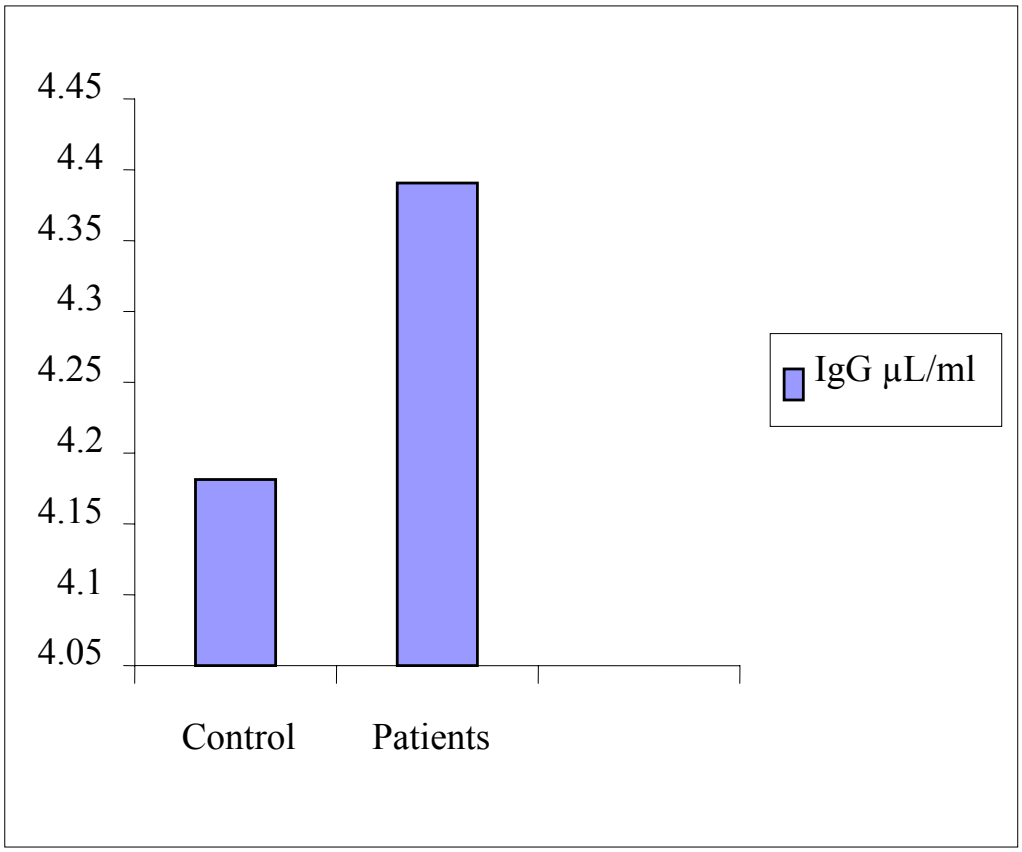
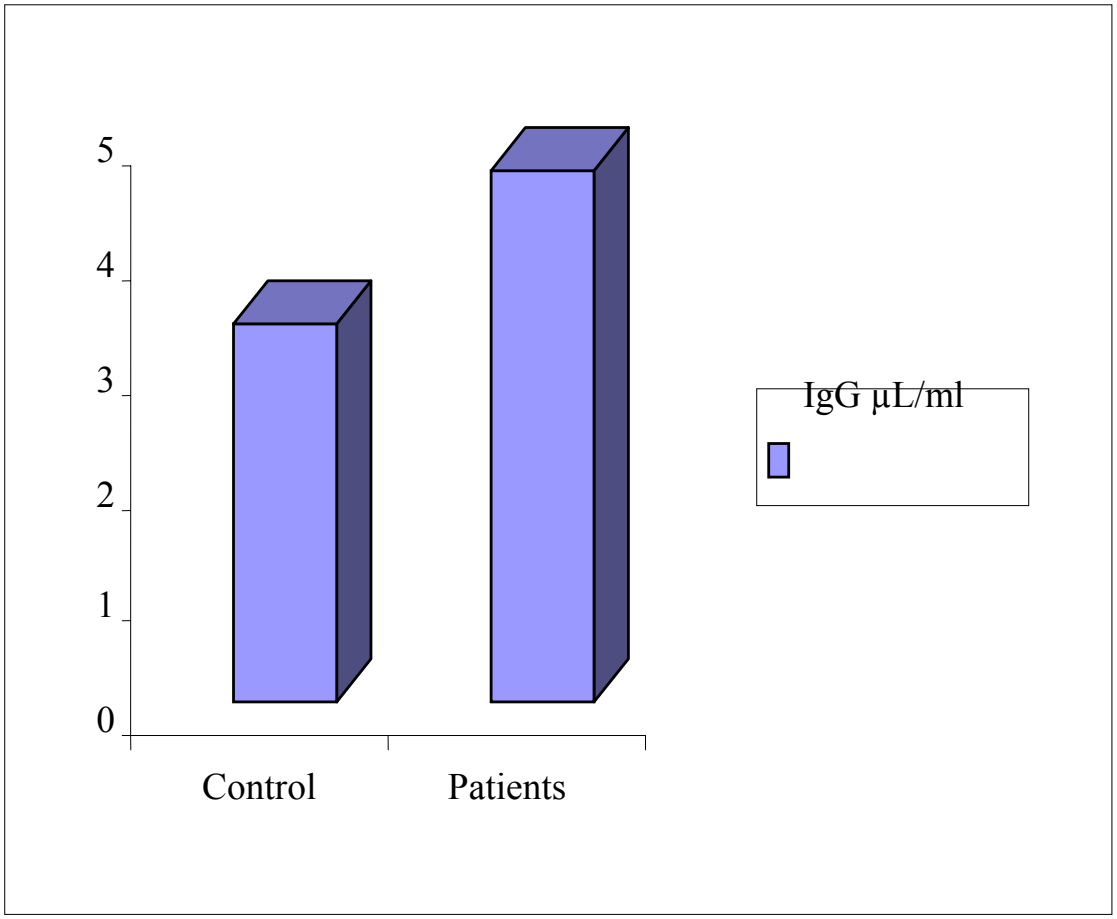


Fig 10: Mean of total IgG in female patients.



3.3 Effects of degree of parasitaemia on serum total proteins, albumin, total globulins and total IgG of patients.

The effects of degree of parasitemia on serum total proteins, albumin, total globulins and total IgG of patients is presented in table (3) Fig. (11, 12).

3.3.1. Total protein

In the present study the level of serum total protein within the same level in one cross patients and control but the two cross patients showed lower level of no significant difference.

3.3.2 Albumin

The levels of albumin showed significantly ($p<0.05$) decreased values in malaria infected patients compared to the control but there is not significantly difference between one cross and two cross patients. However, the lowest albumin level was recorded in patients of two cross parasitaeimia.

3.3.3 Total globulins

In the present study total globulins showed significant ($p < 0.05$) higher values in all malaria patient compared to the control, with slightly higher level in one cross patients compared to two cross patients.

3.3.4 Total IgG

The level of total IgG in all patients showed significant ($p < 0.05$) increases in total IgG in malaria patients compared to the control, but when comparing one cross and two cross patients the level of total IgG showed significantly ($p<0.05$) higher values in patients with one cross compared to patients with two cross parasitaemia.

Table (3): The effect of degree of parasitaemia in serum total proteins, Albumia, total globulin, and total IgG of patients.

Select	Total proteins g/dl	Albumin g/dl	Total globulin g/dl	IgG µL/ml
Control	6.89 ^{a} ± 0.63	4.61 ^{a} ± 0.63	2.28 $_{b}$ ± 0.81	3.73 $_{c}$ ± 0.90
+ patient	6.74 ^{a} ± 0.51	4.12 $_{b}$ ± 0.37	2.61 ^{a} ± 0.64	4.95 ^{a} ± 0.94
++ patient	6.52 ^{a} ± 0.48	4.02 $_{b}$ ± 0.19	2.53 ^{a} ± 0.61	3.94 $_{b}$ ± 0.79

Means within the same columns followed by different letters are significantly different at ($p<0.05$).

+: one cross : (1-10 asexual form of parasites per 100 fields).

++: two cross: (1-99 asexual form of parasites per 100 fields).

Fig 11: The effect of degree of parasitaemia in serum total proteins in patient with *P. falciparum* malaria

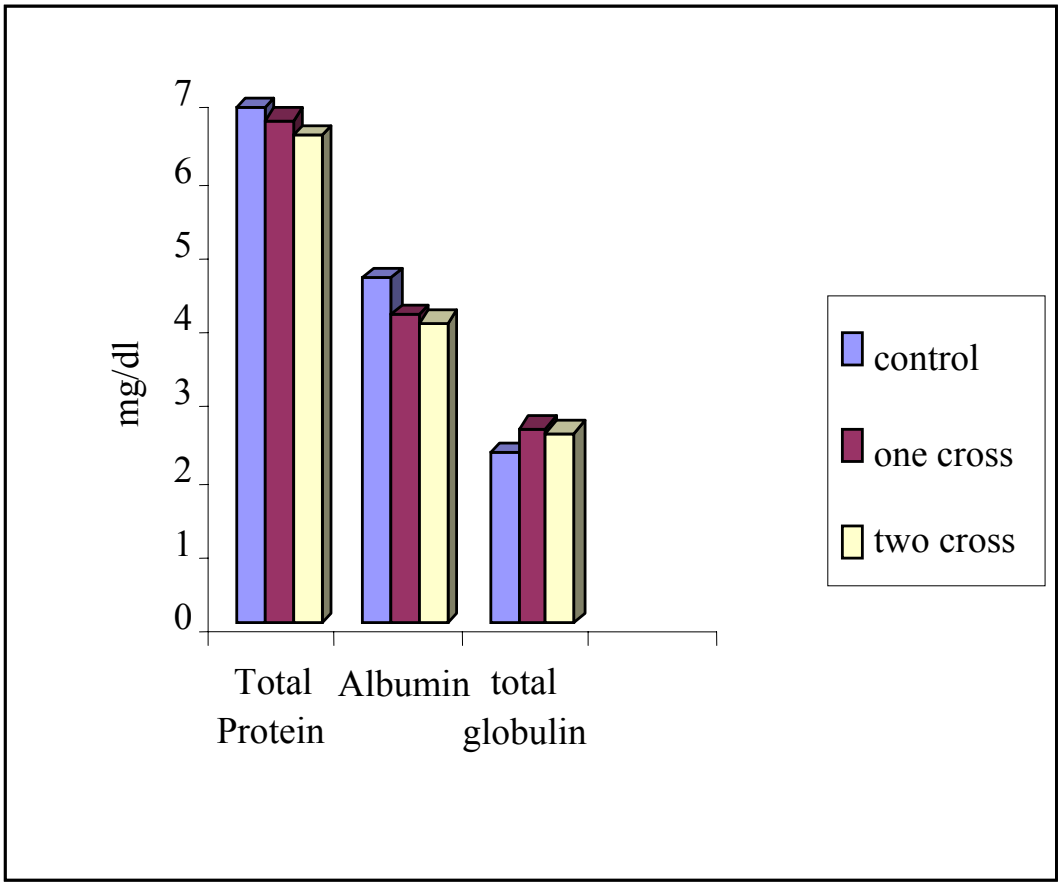
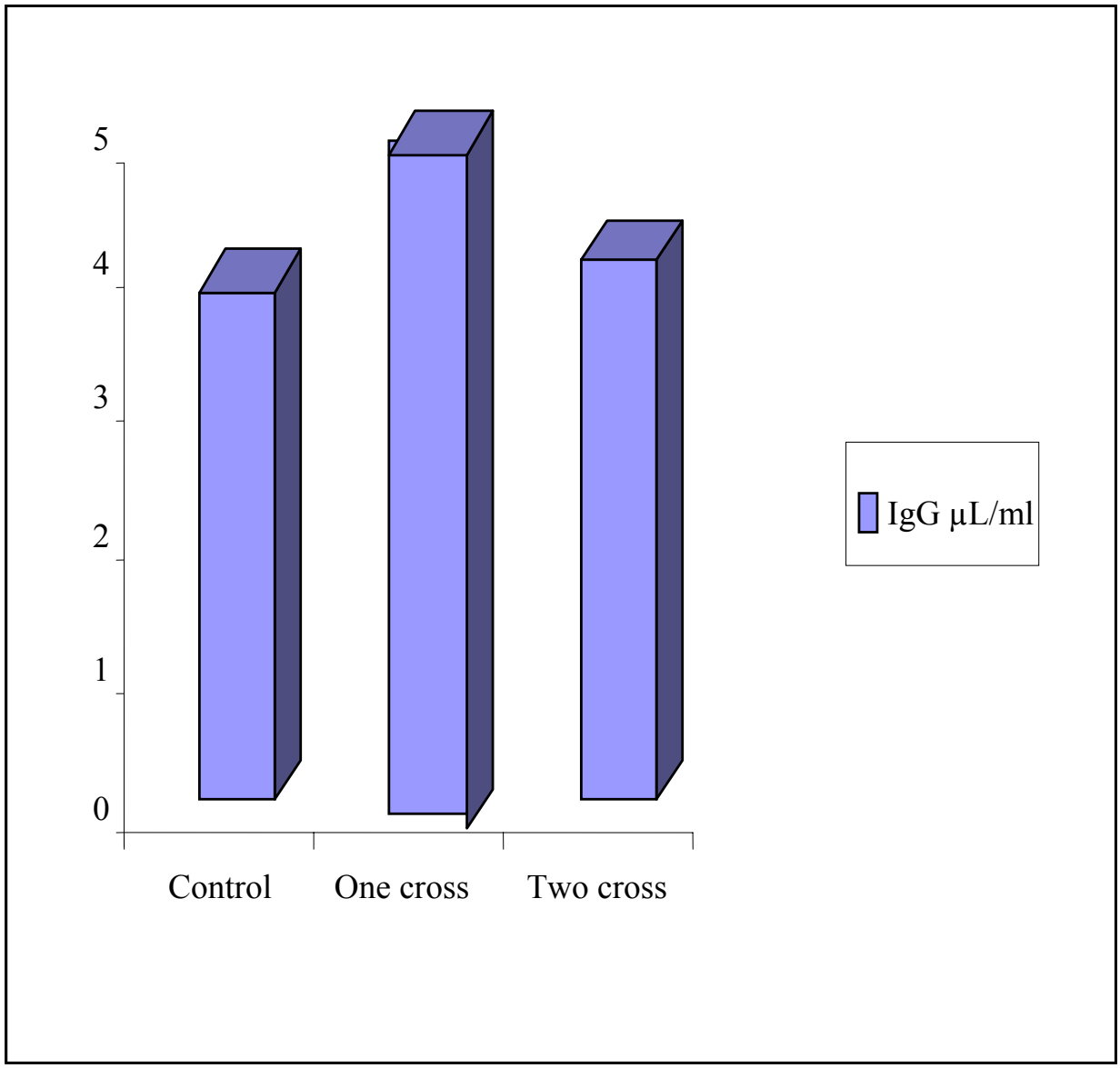


Fig 12: The effect of degree of parasitaemia in serum total IgG in patient with *P. falciparum* malaria



Chapter Four

Discussion

4.1 Effect of malaria on some serum proteins and total IgG in patient with *P. falciparum* malaria.

4.1.1 Total protein

Most disease that alters plasma protein concentrations does so by affecting their volume of distribution or their rates of synthesis, catabolism or excretion. In some patients more than one of these factors may be operating (Smith, *et al,* 1998). Raised plasma total protein concentrations may be due to less of protein free fluid, or excessive stasis during a major increase in one or more of the immunoglobulins. Whereas low plasma total protein concentration may be due to dilution, for example if blood is taken near the site of intravenous infusion, hypoalbuminaemia; or profound immunoglobulins deficiency will be found (Robert *et al*., 1993)

The level of serum total proteins is one of the most factors which is known to be affected by *falciparum* malaria, several studies showed that the level of total protein in serum decrease after the infection with malaria.

(Aluminah, 2000) reported moderate decrease in serum total protein in malaria patients compared to the control subjects, the decrease was suggested to be due to the fact that the concentration of plasma proteins determines the colloids osmotic pressure of plasma and this is influenced by the nutritional status, hepatic and renal function, since malaria has an affect on all functions results in decrease in plasma total protein. In the present study malaria patients showed lower levels of plasma total protein which is in the line with previous studies, though the difference was not significant.

4.1.2 Albumin

In this study the albumin level was found to be significantly ($p< 0.05$) decreased in patients compared to control (table 1) who agree with previous studies. Mishra, (1992) found that serum total protein and albumin were significantly decreased but those were considered more as indicator of acute phase response. Liver cell necrosis was observed in one patient, and oedema and mononuclear cell infiltration in two patients. Though hepatomegaly and mild elevation of enzymes can be observed in a significant proportion of patients, involvement of liver leading to acute hepatitis or liver cell necrosis is a relatively uncommon complication in *P. falciparum* malaria.

4.1.3 Total globulins

Infection with malaria increases the level of total globulins; this result is obtained by previous studies. In the present study the level of total globulins showed significantly ($p< 0.05$) increased values in all malaria infected patients compared to values reported in normal individuals and this result agree with (Abdelgadir, 2002) as she reported elevation in serum total globulin in infected patients compared to non infected school children. These findings coordinate with general idea of acute phase response where the liver shifts from albumin synthesis to acute phase proteins synthesis (Mac Sween and Whaley, 2001).

4.1.4 Total IgG

IgG is the abundant immunoglobulin in the serum of normal humans, it consist of a single immunoglobulin molecule with a sedimentation coefficient of 75 and a molecular weight of 164 K-D .Protective immunity to malaria is difficult to understand but the most often suggested mechanism is the one stated by Baird et al ,(1991), whereby individuals gain an increasing

number of effectors and memory lymphocytes expressing high – affinity antigen-specific receptors that may react with an increasingly large proportion of epitopes occurring on the parasite . In the present study the level of total IgG showed significantly ($p<0.05$) elevated levels in patients compared to non infected individuals and this result agree with (Aribot, 1996 and Rzepezyk, 1997) who reported Significant elevations of IgG3 antibodies in certain populations. However, they reported that elevated concentrations by IgG2 antibodies may also be associated with deceased risk of *P. falciparum* infection. In general, acquisition of active immunity to malaria is slow and requires repeated exposure to the parasite to be maintained.Genetic variability of both the human and the parasite- indused immuno suppression and other reasons account for this instability (Mohan, 1998).

4.2 Effect of sex on serum protein of patient with *P. falciparum* malaria

4.2.1 Total proteins

In previous studies, the values of total proteins in normal individuals showed no differences in males compared to females (Mac Sween, 2001). In the present study also the normal individuals showed no difference in the level of total proteins, when normal males were compared to normal females (Table2).

It is observed in the present study the level of serum proteins is not affected by the sex of the patient but the level of total protein is slightly increase in control compared to patients in both sex . This result was also observed to be significant ($p<0.05$) when only female patients were compared to the normal ones.

4.2.2 Albumin

In the present study males showed significantly ($P< 0.05$) higher difference in normal values of albumin compared to normal females (Table2). But in malaria patients males recorded levels significantly lower ($p<0.05$) than non malaria males whereas females kept the same levels .The effect of malaria on plasma proteins in males was studid previously (Abdalgalil, 2003) and suggested that, the very low levels of total proteins in the infected patients related to develop liver disease, chronic kidney failure, malnutrition, or decrease in the immunoglobulins fractions which is usually a companies malaria.

4.2.3 Total globulins

In the present study there is significantly ($p<0.05$) higher values in total globulins in male malaria patients compared to the healthy individuals, but when males and female were compared, the level of total globulin showed significant ($p<0.05$) high value in female control compared to males. But the level of total globulins in males was significantly ($p<0.05$) increased and reached the same level as the infected females where total globulins levels decreased significantly ($p< 0.05$) when infected with malaria (Table 2) .These findings together with albumin findings showed cleared effects in male patients due to infection with malaria compared to females which influenced to lesser extent by malaria infection.

The high level of total globulins in malaria patients agree with Abdelgadir, (2002) as she reported that the level of total globulins showed significantly ($p< 0.05$) higher values in both sex of malaria infected patients compared to controls with no significant difference in both sex .

4.2.4 Total IgG

The level of total IgG showed significantly ($p< 0.05$) higher levels in malaria infected patients compared to the control. The elevation is suggested to be due to the infection with malaria which leads to increase in total globulins and this result agrees with the previous studies (Aribot, 1996; Rzepezyk, 1997 and Abdelgadir, 2002). The level of total IgG showed significantly ($p<0.05$) higher values in females patients compared to the males.

4.4 Effects of degree of parasitaemia on serum proteins and total IgG.

The effects of degree of parasitaemia on serum total proteins, albumin, total globulins and total IgG of patients is presented in table (3) Fig. (11, 12).

4.3.1. Total protein

In the present study the level of total proteins was within the same levels in patients and control. Abdelgadir, (2002) reported that the level of total protein showed significantly ($p<0.05$) higher level in the patients compared to the control but the level of total proteins was not influenced by the degree of parasitaemia, which in line with present study.

4.3.2 Albumin

In the present study the level of albumin showed significantly ($p<0.05$) decrease in malaria infected patients compared to the control but there is not significantly difference between one cross and two cross patients which agree with Abdalgalil, (2003) who reported that albumin fraction showed lower levels in the two groups of patients, compared to the control individuals and explained his results as that, the pronounced decline in plasma albumin usually known to follow prolonged malnutrition due to

inadequate dietary intake of protein, impaired digestion of protein, chronic loss of protein or inability to synthesize albumin in chronic liver disease.

4.3.3 Total globulins

Cheesbrough, (1998) reported that total globulin levels decreased in heavy malaria cases which can be explained as immunity-suppression which caused by acute infection. In the present study total globulins showed significant ($p < 0.05$) higher values in all malaria patients compared to the control, but the level was not influenced by the degree of parasitaimia (Table 3).This findings is in line with Adalgalil, (2003) who reported that plasma total globulins in the infected and one cross patients were found to be significantly ($p<0.05$) higher compared to the patients with two cross and control individuals. But the level was found to be significantly ($p<0.05$) low in the two cross patients compared to the control. And he suggested that very high levels of total globulins in patients are known to be due to liver disease, acute or chronic infection.

4.3.4 Total IgG

Malaria infection gives rise to host responses, which are regulated by both the innate and adaptive immune systems as well as by environmental factors. Acquired immunity is both specific and stage-specific. It's rarely sterile, but rather associated with low-grade parasitaemia and episodes of the clinical disease throughout life (Marsh, 1992; Trape *et al*, 1994).

(Abdelgadir, 2002) reported an increase of total IgG in all patients compared to control and it increased with the increase of the parasite level. In the present study all patients showed significant ($p < 0.05$) increase in total IgG in malaria patients compare to the control. The elevation is

suggested to be the cause of the increase in total globulins fraction, but when comparing one cross and two cross patients the level of total IgG showed significantly ($p<0.05$) higher values in patient with one cross compared to patients with two cross parasitaemia.The negative correlation between IgG antibodies and parasitaemia described here in agreement with well-established findings by AL-Yaman *et al*,(1996) that antibodies are important for protection by reducing the parasitaemia of malaria .On the other hand elevated concentrations of anti malarial IgG antibodies may reflect not only recent exposure but also previous exposure to parasites and therefore might not necessarily correlate with protection.

Conclusions

From the present study it was concluded that:

1/ The level of serum total proteins and albumin was significantly ($p<0.05$) lower than the control subjects. Whereas the mean values of total globulins and IgG showed significantly ($p< 0.05$) higher value compared to the healthy individuals.

2 / The level of serum proteins is not affected by the sex whereas albumin fraction in males showed significantly ($P< 0.05$) higher difference in normal males compared to normal females.

The level of total total IgG showed significantly ($P< 0.05$) increased in female patients compared to male patients.

3 / Proteins levels is not influenced by the degree of parasitaemia whereas the level of albumin showed significantly ($p<0.05$) decrease in malaria infected patients compared to the control .But there is no significant difference between one cross and two cross patients.

4 / The level of IgG showed significantly ($p<0.05$) elevated levels in patients compared to non infected individuals also there is significant ($p<0.05$) increased between one cross and two cross patients.

From the above the following recommendations were offered:

1/ As this study showed that the result of IgG in female patients was highly significant ($p<0.05$) comparined with male patients, therefore .It is recommended to use the immunological techniques, to clarify the reasons of increasing and decreasing of IgG antibodies in the malaria patients.

2/ There is a need for testing more patients for better understanding of immunity against malaria and to determine the effect of sex on the level of serum proteins.

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Appendices

Table 4: The infection of malaria in EL –Duiem province during (January 2000 - October 2005).

Years	In patient	Out patient
2000	708	3432
2001	2990	5428
2002	I669	4511
2003	1503	2988
2004	1344	3209
2005	1161	3768

Fig 13: The infection of malaria in EL-Duiem province during (January 2000 –October 2005).